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(54) **Novel variant of the cellular receptor LXR alpha**

(57) The present invention provides, *inter alia*, a novel nuclear receptor protein. In a preferred embodiment of the invention a novel LXR- α homologue is provided for. Also provided are the nucleic acid sequence

encoding this novel nuclear receptor protein, as well as compounds and methods for using this protein and its nucleic acid sequence.

Description

BACKGROUND OF THE INVENTION

[0001] Multicellular organisms are dependent on advanced mechanisms of information transfer between cells and body compartments. The information that is transmitted can be highly complex and can result in the alteration of genetic programs involved in cellular differentiation, proliferation, or reproduction. The signals, or hormones, are often simple molecules, such as peptides, fatty acid, or cholesterol derivatives.

[0002] Many of these signals produce their effects by ultimately changing the transcription of specific genes. One well-studied group of proteins that mediate a cell's response to a variety of signals is the family of transcription factors known as nuclear receptors, hereinafter referred to often as "NR". Members of this group include receptors for steroid hormones, vitamin D, ecdysone, cis and trans retinoic acid, thyroid hormone, bile acids, cholesterol-derivatives, fatty acids (and other peroxisomal proliferators), as well as so-called orphan receptors, proteins that are structurally similar to other members of this group, but for which no ligands are known (Escriva, H. et al., Ligand binding was acquired during evolution of nuclear receptors, PNAS, 94, 6803 - 6808, 1997). Orphan receptors may be indicative of unknown signaling pathways in the cell or may be nuclear receptors that function without ligand activation. The activation of transcription by some of these orphan receptors may occur in the absence of an exogenous ligand and/or through signal transduction pathways originating from the cell surface (Mangelsdorf, D. J. et al., The nuclear receptor superfamily: the second decade, Cell 83, 835-839, 1995).

[0003] In general, three functional domains have been defined in NRs. An amino terminal domain is believed to have some regulatory function. A DNA-binding domain hereinafter referred to as "DBD" usually comprises two zinc finger elements and recognizes a specific Hormone Responsive Element hereinafter referred to as "HRE" within the promoters of responsive genes. Specific amino acid residues in the "DBD" have been shown to confer DNA sequence binding specificity (Scheda, M. & Yamamoto, K.R., Mammalian Glucocorticoid Receptor Derivatives Enhance Transcription in Yeast, Science, 241:965-967, 1988). A Ligand-binding-domain hereinafter referred to as "LBD" is at the carboxy-terminal region of known NRs. In the absence of hormone, the LBD appears to interfere with the interaction of the DBD with its HRE.

[0004] Hormone binding seems to result in a conformational change in the NR and thus opens this interference (Brzozowski et al., Molecular basis of agonism and antagonism in the oestrogen receptor, Nature, 389, 753 - 758, 1997; Wagner et al., A structural role for hormone in the thyroid hormone receptor, Nature, 378, 690 - 697, 1995). A NR without the hormone binding domain (herein referred to as "HBD") constitutively activates transcription but at a low level.

[0005] Both the amino-terminal domain and the LBD appear to have transcription activation functions hereinafter referred to as "TAF". Acidic residues in the amino-terminal domains of some nuclear receptors may be important for these transcription factors to interact with RNA polymerase. TAF activity may be dependent on interactions with other protein factors or nuclear components (Diamond et al., Transcription Factor Interactions: Selectors of Positive or Negative Regulation from a Single DNA Element, Science, 249:1266-1272, 1990). Certain onco-proteins (e.g., c-Jun and c-Fos) can show synergistic or antagonistic activity with glucocorticoid receptors (GR) in transfected cells. Furthermore, the receptors for estrogen and vitamins A and D, and fatty acids have been shown to interact, either physically or functionally, with the Jun and Fos components of AP-1 in the transactivation of steroid- or AP-1 regulated genes.

[0006] Coactivators or transcriptional activators are proposed to bridge between sequence specific transcription factors, the basal transcription machinery and in addition to influence the chromatin structure of a target cell. Several proteins like SRC-1, ACTR, and Grip1 interact with NRs in a ligand enhanced manner (Heery et al., A signature motif in transcriptional coactivators mediates binding to nuclear receptors, Nature, 387, 733 - 736; Heinzel et al., A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression, Nature 387, 43 - 47, 1997). Furthermore, the physical interaction with negative receptor-interacting proteins or corepressors has been demonstrated (Xu et al., Coactivator and Corepressor complexes in nuclear receptor function, Curr Opin Genet Dev, 9 (2), 140 - 147, 1999).

[0007] Nuclear receptor modulators like steroid hormones affect the growth and function of specific cells by binding to intracellular receptors and forming nuclear receptor-ligand complexes. Nuclear receptor-hormone complexes then interact with a hormone response element (HRE) in the control region of specific genes and alter specific gene expression.

[0008] Over the past decade, new members of the nuclear hormone gene family have been identified that lack known ligands. These orphan receptors can be used to uncover signaling molecules that regulate yet unidentified physiological networks. Some of these orphan receptors are constitutively active in transactivate target genes without the need to interact with a ligand (Mangelsdorf et al., 1995).

[0009] US 5,747,661, and Willy et al. (Willy P.J, et al. LXR, a nuclear receptor that defines a distinct retinoid response pathway. Genes Dev 1995 May 1;9(9):1033-45) describe the characterization of LXR alpha as an orphan member of

the nuclear hormone receptor superfamily that can function as a tissue-specific mediator of 9-cis retinoic acid (9cRA). When expressed in cells, LXR alpha activates transcription in response to 9cRA on a distinct response element, termed an LXRE. Significantly, neither RXR homodimers nor RXR/RAR heterodimers are able to substitute for LXR alpha in mediating this retinoid response. The LXR alpha response to retinoids is described as due to its unique interaction with endogenous RXR. This interaction shifts RXR from its previously described role as a silent, heterodimer partner to an active ligand binding partner, thus defining a novel retinoid response pathway.

[0010] Furthermore, US 5,932,699 discloses a method for determining whether a test protein is capable of interacting with a retinoid X receptor protein. The method involves: (a) providing a host cell which contains (i) a reporter gene operably linked to a protein binding site; (ii) a first fusion gene which expresses a first fusion protein, the first fusion protein including a retinoid X receptor protein covalently bonded to a binding moiety which is capable of specifically binding to the protein binding site; and (iii) a second fusion gene which expresses a second fusion protein, the second fusion protein including the test protein covalently bonded to a gene activating moiety; and (b) determining whether the test protein increases expression of the reporter gene as an indication of its ability to interact with the retinoid X receptor protein. Also disclosed is purified DNA encoding retinoid X receptor-interacting proteins and the polypeptides expressed from such DNA.

[0011] Janowski et al. (Janowski BA, Willy PJ, Devi TR, Falck JR, Mangelsdorf DJ. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. *Nature* 1996 Oct 24;383(6602):728-31) demonstrate the existence of a nuclear receptor signalling pathway for oxysterols and suggest that LXR alpha may be important as a sensor of cholesterol metabolites.

[0012] Finally, Fujita et al. (Fujita A, Mitsuhashi T. Differential regulation of ligand-dependent and ligand-independent functions of the mouse retinoid X receptor beta by alternative splicing.

[0013] *Biochem Biophys Res Commun* 1999 Feb 24;255(3):625-30) describe the functions of a unique isoform of mouse RXRbeta2 (mRXRbeta2E) which has an in-frame four-amino-acid insertion in the ligand binding domain. mRXRbeta2E formed heterodimers with TR, VDR, RAR, and LXR, and enhanced transcriptional activity of the non-permissive VDR to the same extent as mRXRbeta2. With the permissive LXR, mRXRbeta2E did not show 9-cis RA-dependent transactivation and inhibited mRXRbeta2 activity. Thus, although mRXRbeta2E can perform ligand-independent functions of RXR, it cannot mediate the ligand-dependent functions of RXR, which indicates that alternative splicing plays an important role in the differential regulation of these two important functions of RXR. Nevertheless, an alternative splicing of LXR has not been described or proposed by the above-mentioned references.

[0014] Through its regulatory function in cholesterol metabolism, an additional LXR- α homologue could serve as a target for cholesterol lowering drugs and exert beneficial effects in diseases like arteriosclerosis and other metabolic disorders. Furthermore, since not all variants of LXR alpha are known yet, it was impossible to elucidate the effects of LXR alpha modulating substances on the individual isoforms of the receptor, thus making a reliable prediction impossible.

[0015] It was thus an object of the present invention to provide for a novel LXR nuclear receptor. In a preferred embodiment of the invention it was an object to provide for a homologue of LXR- α . It was an object of the present invention to provide for means of producing this receptor as well as means of screening for agonists and antagonists to the receptor. Further objects of the invention are outlined below.

SUMMARY OF THE INVENTION

[0016] The present invention provides, *inter alia*, a novel nuclear receptor protein. In a preferred embodiment of the invention a novel LXR- α homologue is provided for. Also provided is the nucleic acid sequence encoding this novel nuclear receptor protein, as well as compounds and methods for using this protein and its nucleic acid sequence.

[0017] The present invention provides a novel proteins, nucleic acids, and methods useful for developing and identifying compounds for the treatment of such diseases and disorders as metabolic disorders, immunological indications, hormonal dysfunctions, neurosystemic diseases and in preferred embodiments, high cholesterol and arteriosclerosis as well as other metabolic disorders.

[0018] Identified and disclosed herein is the protein sequence for a novel nuclear receptor and the nucleic acid sequence encoding this nuclear receptor, which is designated herein as "LXR- α 3 nuclear receptor" or simply "LXR- α 3 receptor", or "LXR- α 3".

[0019] The importance of this discovery is manifested in the effects of LXR- α 3 to modulate genes involved in cellular functions like regulation of metabolism and cell homeostasis, cell proliferation and differentiation, pathological cellular aberrations, or cellular defense mechanisms including tumor development, *i.e.* cancer.

[0020] Thus, this LXR- α 3 protein is useful for screening for LXR- α 3 agonists and antagonist activity for controlling these conditions. Interestingly, the receptor according to the present invention lacks the DNA-binding domain, which is present in the "regular" LXR nuclear receptor. Thus, this present receptor will behave surprisingly different in terms of pairing, interaction with other proteins (e.g. RXR) and interacting compounds (ligands), and therefore its biological

function. Thus, this receptor will enable a whole new area of applications with respect to the development and identification of compounds for the treatment of such diseases and disorders as metabolic disorders, immunological indications, hormonal dysfunctions, neurosystemic diseases and in preferred embodiments, high cholesterol and arteriosclerosis as well as other metabolic disorders.

[0021] In one aspect of the present invention, we provide isolated nucleic acid sequences for a novel receptor, the LXR- α 3 receptor. In particular, we provide the cDNA sequences, protein sequences as well as the genomic sequences encoding the human LXR- α 3 receptor.

[0022] These nucleic acid sequences have a variety of uses. For example, they are useful for making vectors and for transforming cells, both of which are ultimately useful for production of the LXR- α 3 protein.

[0023] They are also useful as scientific research tools for developing nucleic acid probes for determining LXR- α 3 expression levels, e.g., to identify diseased or otherwise abnormal states. They are useful for developing analytical tools such as anti sense oligonucleotides for selectively inhibiting expression of the LXR- α 3 gene to determine physiological responses.

[0024] In another aspect of the present invention, we provide a homogenous composition comprising the LXR- α 3 protein. The protein is useful for screening drugs for agonist and antagonist activity, and, therefore, for screening for drugs useful in regulating physiological responses associated with LXR- α 3. Specifically, antagonists to the LXR- α 3 receptor could be used to treat diseases and disorders as metabolic disorders, immunological indications, hormonal dysfunctions, neurosystemic diseases and in preferred embodiments, high cholesterol and arteriosclerosis as well as other metabolic disorders, whereas agonists could be used for the treatment of these conditions. The proteins are also useful for developing antibodies for detection of the protein.

[0025] Flowing from the foregoing are a number of other aspects of the invention, including (a) vectors, such as plasmids, comprising the LXR- α 3 nuclear receptor nucleic acid sequence that may further comprise additional regulatory elements, e.g., promoters, (b) transformed cells that express the LXR- α 3, (c) nucleic acid probes, (d) antisense oligonucleotides, (e) agonists, (f) antagonists, and (g) transgenic mammals. Further aspects of the invention comprise methods for making and using the foregoing compounds and compositions.

[0026] The foregoing merely summarizes certain aspects of the present invention and is not intended, nor should it be construed, to limit the invention in any manner. All patents and other publications recited herein are hereby incorporated by reference in their entirety.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

THE LXR- α 3 PROTEIN AND NUCLEIC ACID:

[0027] The present invention comprises a novel member of the LXR nuclear receptor superfamily which the inventors herein refer to as LXR- α 3. Particularly preferred embodiments of the LXR- α 3 receptor are those having an amino acid sequence substantially the same as SEQ ID NO. 2. The analysis of the amino acid sequence confirms that the present protein is indeed a member of the nuclear receptor family which is closely related to LXR. Amongst other features, the present variant lacks the DNA-binding domain present in the LXR receptors known to date.

[0028] In view of the above, a major aspect of the present invention is the provision of a polypeptide of LXR- α 3 receptor, comprising the amino acid sequence according to SEQ ID No. 2, or having an amino acid sequence substantially the same or similar as SEQ ID No. 2.

[0029] As used herein, a protein "having an amino acid sequence substantially the same or similar as SEQ ID NO x" (where "x" is the number of one of the protein sequences recited in the Sequence Listing) means a protein whose amino acid sequence is the same as SEQ ID NO x or differs only in a way such that at least 50% of the residues compared in a sequence alignment with SEQ ID NO. x are identical, preferably 75% of the residues are identical, even more preferably 95% of the residues are identical and most preferably at least 98% of the residues are identical.

[0030] Those skilled in the art will appreciate that conservative substitutions of amino acids can be made without significantly diminishing the protein's affinity for interacting proteins, DNA binding sites, LXR- α 3 receptor modulators, e.g. small molecular hydrophobic compounds, or RNA.

[0031] Other substitutions may be made that increase the protein's affinity for these compounds. Making and identifying such proteins is a routine matter given the teachings herein, and can be accomplished, for example, by altering the nucleic acid sequence encoding the protein (as disclosed herein), inserting it into a vector, transforming a cell, expressing the nucleic acid sequence, and measuring the binding affinity of the resulting protein, all as taught herein.

[0032] Another aspect of the present invention is directed to a fusion protein comprising a polypeptide according to the present invention. Such fusion proteins are described in more detail below. In addition, the polypeptide according to the present invention can be a synthetically produced polypeptide. Again, the way of producing such polypeptides is further described below.

[0033] As used herein, if reference to LXR- α 3, the LXR- α 3 receptor, the nuclear receptor LXR- α 3 or the LXR- α 3

nuclear receptor is made it is meant as a reference to any protein having an amino acid sequence substantially the same as SEQ ID NO.: 2.

[0034] The LXR- α 3 receptor proteins provided by the present invention are of mammalian, more preferably human origin.

[0035] The nucleic acids claimed according to the present invention may be present in various forms, i.e. as an RNA, DNA, cDNA or as genomic DNA. In particular, the present invention is related to a nucleic acid encoding for a polypeptide according to the invention, wherein said nucleic acid is genomic DNA, cDNA, immature mRNA or mature mRNA, in particular the nucleic acid sequence according to SEQ ID No. 1, and optionally comprises at least one suitable label. Furthermore, the sequence of the inventive nucleic acid(s) can comprises at least one intron and/or a polyA-sequence.

According to another aspect of the present invention, the nucleic acid according to invention can be present in form of its complementary antisense-sequence. Preferably, the nucleic acid is a synthetically produced nucleic acid.

[0036] The present invention also comprises nucleic acid sequences encoding the LXR- α 3 receptor, which nucleic acid sequences are substantially the same as SEQ ID NO. 1 or splice variants thereof, their sequence complements or complements of said splice variants SEQ ID NO. 1 encodes a human cDNA LXR- α 3 receptor variant and is the preferred embodiment of the present invention.

[0037] Herein the "complement" refers to the complementary strand of the nucleic acid according to the invention, thus the strand that would hybridise to the nucleic acid according to the invention. In accordance with standard biological terminology all DNA sequences herein are however written in 5'-3' orientation, thus the if a complement is mentioned (see also figures) it is actually a "reverse" complement (as also stated in the figures). For simplification purposes they may however sometimes be referred to simply as "complements".

[0038] As used herein the term "a molecule having a nucleotide sequence substantially the same as SEQ ID NO y" (wherein "y" is the number of one of the protein-encoding nucleotide sequences listed in the Sequence Listing) means a nucleic acid encoding a protein "having an amino acid sequence substantially the same as SEQ ID NO y+1" (wherein "y+1" is the number of the amino acid sequence for which nucleotide sequence "y" codes) as defined above. This definition is intended to encompass natural allelic variations in the LXR- α 3 sequence. Cloned nucleic acid provided by the present invention may encode LXR- α 3 protein of any species of origin, including (but not limited to), for example, mouse, rat, rabbit, cat, dog, primate, and human.

IDENTIFICATION OF VARIANTS AND HOMOLOGUES AS WELL AS USE OF PROBES:

[0039] Nucleic acid hybridisation probes provided by the invention are nucleic acids consisting essentially of the nucleotide sequences complementary to any sequence depicted in SEQ ID NO. 1 or a part thereof and that are effective in nucleic acid hybridisation

[0040] Nucleic acid hybridisation probes provided by the invention are nucleic acids capable of detecting i.e. hybridising to the gene encoding the polypeptides according to SEQ ID NO. 1.

[0041] Nucleic acid probes are useful for detecting LXR- α 3 gene expression in cells and tissues using techniques well-known in the art, including, but not limited to, Northern blot hybridisation, in situ hybridisation, and Southern hybridisation to reverse transcriptase - polymerase chain reaction product DNAs. The probes provided by the present invention, including oligonucleotide probes derived therefrom, are also useful for Southern hybridisation of mammalian, preferably human, genomic DNA for screening for restriction fragment length polymorphism (RFLP) associated with certain genetic disorders. As used herein, the term complementary means a nucleic acid having a sequence that is sufficiently complementary in the Watson-Crick sense to a target nucleic acid to bind to the target under physiological conditions or experimental conditions those skilled in the art routinely use when employing probes.

[0042] It is understood in the art that a nucleic acid sequence will hybridise with a complementary nucleic acid sequence under high stringent conditions as defined herein, even though some mismatches may be present. Such closely matched, but not perfectly complementary sequences are also encompassed by the present invention. For example, differences may occur through genetic code degeneracy, or by naturally occurring or man made mutations and such mismatched sequences would still be encompassed by the present claimed invention.

[0043] Preferably, the nucleotide sequence of the nuclear receptor LXR- α 3 (SEQ ID NO. 1 or splice variants thereof) and/or their complements can be used to derive oligonucleotide fragments (probes) of various length. If the probe is used to detect a human LXR- α 3 sequence most preferably a complement of SEQ ID NO. 1 is used. If the probe is supposed to detect a human, mouse or a rodent sequence probes complementary to SEQ ID NO. 1, or their respective complements are preferred. Stretches of 17 to 30 nucleotides are used frequently but depending on the screening parameters longer sequences as 40, 50, 100, 150 up to the full length of the sequence may be used. Those probes can be synthesised chemically and are obtained readily from commercial oligonucleotide providers. Chemical synthesis has improved over the years and chemical synthesis of oligonucleotides as long as 100-200 bases is possible. The field might advance further to allow chemical synthesis of even longer fragments. Alternatively, probes can also be obtained by biochemical *de novo* synthesis of single stranded DNA. In this case the nucleotide sequence of the nuclear

receptor LXR- α 3 or its complement serve as a template and the corresponding complementary strand is synthesised. A variety of standard techniques such as nick translation or primer extension from specific primers or short random oligonucleotides can be used to synthesise the probe (Molecular Cloning: A Laboratory Manual (3 Volume Set) by Joseph Sambrook, David, W. Russell, Joe Sambrook, 2100 pages 3rd edition (January 15, 2001; . Molecular cloning: a laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, 1989)). Nucleic acid reproduction technologies exemplified by the polymerase chain reaction (Saiki, R.K. et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239, 487-491 (1988)) are commonly applied to synthesise probes. In the case of techniques using specific primers the nucleic acid sequence of the nuclear receptor LXR- α 3 or its complement is not only used as a template in the biochemical reaction but also to derive the specific primers, which are needed to prime the reaction.

[0044] In some cases one might also consider to use the nucleic acid sequence of the cofactor or its complement as a template to synthesise an RNA probe. A promoter sequence for a DNA-dependent RNA polymerase has to be introduced at the 5'-end of sequence. As an example this can be done by cloning the sequence into a vector which carries the respective promoter sequence. It is also possible to introduce the needed sequence by synthesising a primer with the needed promoter in the form of a 5' "tail". The chemical synthesis of a RNA probe is another option.

[0045] Appropriate means are available to detect the event of a hybridisation. There is a wide variety of labels and detection systems, e.g. radioactive isotopes, fluorescent, or chemiluminescent molecules which can be linked to the probe. Furthermore, there are methods of introducing haptens which can be detected by antibodies or other ligands such as the avidin/biotin high affinity binding system.

[0046] Hybridisation can take place in solution or on solid phase or in combinations of the two, e.g. hybridisation in solution and subsequent capture of the hybridisation product onto a solid phase by immobilised antibodies or by ligand coated magnetic beads.

[0047] Hybridisation probes act by forming selectively duplex molecules with complementary stretches of a sequence of a gene or a cDNA. The selectivity of the process can be controlled by varying the conditions of hybridisation. To select sequences which are identical highly homologous to the sequence of interest stringent conditions for the hybridisation will be used, e.g. low salt in the range of 0.02 M to 0.15 M salt and/or high temperatures in the range from 50°C degrees centigrade to 70°C degrees centigrade. Stringency can be further improved by the addition of formamide to the hybridisation solution. The use of stringent conditions which means that only little mismatch or a complete match will lead to a hybridisation product would be used to isolate closely related members of the same gene family. Thus, as used herein stringent hybridisation conditions are those where between 0.02 M to 0.15 M salt and/or high temperatures in the range from 50°C degrees centigrade to 70°C degrees centigrade are applied.

[0048] The use of highly stringent conditions or conditions of "high stringency" means that only very little mismatch or a complete match which lead to a hybridisation product would be used to isolate very closely related members of the same gene family. Thus, as used herein highly stringent hybridisation conditions are those where between 0.02 - 0.3 M salt and 65°C degrees centigrade are applied for about 5 to 18 hours of hybridisation time and additionally, the sample filters are washed twice for about 15 minutes each at between 60°C - 65°C degrees centigrade, wherein the first washing fluid contains about 0.1 M salt (NaCl and/or Sodium Citrate) and the second contains only about 0.02 M salt (NaCl and/or Sodium Citrate). In a preferred embodiment the following conditions are considered to be highly stringent:

[0049] Hybridisation in a buffer containing 2 x SSC (0.03 M Sodium Citrate, 0.3 M NaCl at 65°C-68°C degrees centigrade for 12 hours, followed by a washing step for 15 minutes in 0.5 x SSC, 0.1% SDS, and a washing step for 15 minutes at 65°C degrees centigrade in 0.1 x SSC, 0.1% SDS.

[0050] Less stringent hybridization conditions, e.g. 0.15 M salt - 1 M salt and/or temperatures from 22°C degrees centigrade to 56°C degrees centigrade are applied in order to detect functionally equivalent genes in the same species or for orthologous sequences from other species.

[0051] Unspecific hybridization products are removed by washing the reaction products repeatedly in 2 x SSC solution and increasing the temperature.

DEGENERATE PCR AND CLONING OF HOMOLOGUES

[0052] The nucleotide sequence of the nuclear receptor LXR- α 3 or its complement can be used to design primers for a polymerase chain reaction. Due to the degeneracy of the genetic code the respective amino acid sequence is used to design oligonucleotides in which varying bases coding for the same amino acid are included. Numerous design rules for degenerate primers have been published (Compton et al, 1990). As in hybridization there are a number of factors known to vary the stringency of the PCR. The most important parameter is the annealing temperature. To allow annealing of primers with imperfect matches annealing temperatures are often much lower than the standard annealing temperature of 55°C, e.g. 35°C to 52°C degrees can be chosen. PCR reaction products can be cloned. Either the PCR product is cloned directly, with reagents and protocols from commercial manufacturers (e.g. from Invitrogen, San Diego,

USA). Alternatively, restriction sites can be introduced into the PCR product via a 5'-tail of the PCR primers and used for cloning. Primers for the amplification of the entire or partial pieces of the LXR- α 3 gene or mRNA, or for reverse transcription may be designed making use of the sequences according to the invention, i.e. those depicted in the figures below.

GENETIC VARIANTS

[0053] Fragments from the nucleotide sequence of the nuclear receptor LXR- α 3 (SEQ ID NO. 1) or their complements can be used to cover the whole sequence with overlapping sets of PCR primers. Also the genomic sequences may be used. These primers are used to produce PCR products using genomic DNA from a human diversity panel of healthy individuals or genomic DNA from individuals which are phenotypically conspicuous. Also the genomic sequences may be used, i.e. that of the human clone. The PCR products can be screened for polymorphisms, for example by denaturing gradient gel electrophoresis, binding to proteins detecting mismatches or cleaving heteroduplexes or by denaturing high-performance liquid chromatography. Products which display mutations need to be sequenced to identify the nature of the mutation. Alternatively, PCR products can be sequenced directly omitting the mutation screening step to identify genetic polymorphisms. If genetic variants are identified and are associated with a discrete phenotype, these genetic variations can be included in diagnostic assays. The normal variation of the human population is of interest in designing screening assays as some variants might interact better or worse with a respective lead substance (a pharmacodynamic application). Polymorphisms or mutations which can be correlated to phenotypic outcome are a tool to extend the knowledge and the commercial applicability of the nucleotide sequence of the nuclear receptor LXR- α 3 or its complement or their gene product, as variants might have a slightly different molecular behavior or desired properties. Disease-causing mutations or polymorphisms allow the replacement of this disease inducing gene copy with a wild-type copy by means of gene therapy approaches and/or the modulation of the activity of the gene product by drugs.

PREPARATION OF POLYNUCLEOTIDES:

[0054] DNA which encodes receptor LXR- α 3 may be obtained, in view of the instant disclosure, by chemical synthesis, by screening reverse transcripts of mRNA from appropriate cells or cell line cultures, by screening genomic libraries from appropriate cells, or by combinations of these procedures, as illustrated below.

[0055] Screening of mRNA or genomic DNA may be carried out with oligonucleotide probes generated from the LXR- α 3 nucleotide sequences information provided herein.

[0056] Probes may be labelled with a detectable group such as a fluorescent group, a radioactive atom or a chemiluminescent group in accordance with known procedures and used in conventional hybridisation assays, as described in greater detail in the Examples below. Alternatively, the LXR- α 3 nucleotide sequence may be obtained by use of the polymerase chain reaction (PCR) procedure, with the PCR oligonucleotide primers being produced from the LXR- α 3 nucleotide sequences provided herein.

[0057] Upon purification or synthesis, the nucleic acid according to the invention may be labelled, e.g. for use as a probe.

[0058] As single and differential labelling agents and methods, any agents and methods which are known in the art can be used. For example, single and differential labels may consist of the group comprising enzymes such as β -galactosidase, alkaline phosphatase and peroxidase, enzyme substrates, coenzymes, dyes, chromophores, fluorescent, chemiluminescent and bio-luminescent labels such as FITC, Cy5, Cy5.5, Cy7, Texas-Red and IRD40 (Chen et al. (1993), J. Chromatog. A 652: 355-360 and Kambara et al. (1992), Electrophoresis 13: 542-546), ligands or haptens such as biotin, and radioactive isotopes such as ^3H , ^{35}S , ^{32}P , ^{125}I and ^{14}C .

EXPRESSION OF THE LXR- α 3 PROTEIN/POLYPEPTIDE:

[0059] The nuclear receptor LXR- α 3 nucleic acid or polypeptide may be synthesised in host cells transformed with a recombinant expression construct comprising a nucleic acid encoding the nuclear receptor LXR- α 3.

[0060] Such a recombinant expression construct can also be comprised of a vector that is a replicable DNA construct.

[0061] Amplification vectors do not require expression control domains. All that is needed is the ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants. See, Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd Edition, Cold Spring Harbor Press, New York, 1989).

[0062] An expression vector comprises a polynucleotide operatively linked to a prokaryotic promoter. Alternatively, an expression vector is a polynucleotide operatively linked to an enhancer-promoter that is a eukaryotic promoter, and the expression vector further has a polyadenylation signal that is positioned 3' of the carboxy-terminal amino acid and within a transcriptional unit of the encoded polypeptide. A promoter is a region of a DNA molecule typically within about

500 nucleotide pairs in front of (upstream of) the point at which transcription begins (*i.e.*, a transcription start site). In general, a vector contains a replicon and control sequences which are derived from species compatible with the host cell. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells.

[0063] Another type of discrete transcription regulatory sequence element is an enhancer. An enhancer provides specificity of time, location and expression level for a particular encoding region (e.g., gene). A major function of an enhancer is to increase the level of transcription of a coding sequence in a cell.

[0064] As used herein, the phrase "enhancer-promoter" means a composite unit that contains both enhancer and promoter elements. An enhancer-promoter is operatively linked to a coding sequence that encodes at least one gene product.

[0065] An enhancer-promoter used in a vector construct of the present invention may be any enhancer-promoter that drives expression in a prokaryotic or eukaryotic cell to be transformed/transfected.

[0066] A coding sequence of an expression vector is operatively linked to a transcription terminating region. RNA polymerase transcribes an encoding DNA sequence through a site where polyadenylation occurs.

[0067] An expression vector comprises a polynucleotide that encodes a nuclear receptor LXR- α 3 polypeptide. Such a polynucleotide is meant to include a sequence of nucleotide bases encoding a nuclear receptor LXR- α 3 polypeptide sufficient in length to distinguish said segment from a polynucleotide segment encoding a non- nuclear receptor LXR- α 3 polypeptide.

[0068] A polypeptide of the invention may also encode biologically functional polypeptides or peptides which have variant amino acid sequences, such as with changes selected based on considerations such as the relative hydropathic score of the amino acids being exchanged.

[0069] These variant sequences are those isolated from natural sources or induced in the sequences disclosed herein using a mutagenic procedure such as site-directed mutagenesis.

[0070] Furthermore, an expression vector of the present invention may contain regulatory elements for optimised translation of the polypeptide in prokaryotic or eukaryotic systems. These sequences are operatively located around the transcription start site and are most likely similar to ribosome recognition sites like prokaryotic ribosome binding sites (RBS) or eukaryotic Kozak sequences as known in the art (Kozak M., Initiation of translation in prokaryotes and eukaryotes. *Gene* 234, 187-208 (1999)).

[0071] An expression vector of the present invention is useful both as a means for preparing quantities of the nuclear receptor LXR- α 3 polypeptide-encoding DNA itself, and as a means for preparing the encoded nuclear receptor LXR- α 3 polypeptide and peptides. It is contemplated that where nuclear receptor LXR- α 3 polypeptides of the invention are made by recombinant means, one may employ either prokaryotic or eukaryotic expression vectors as shuttle systems.

[0072] Where expression of recombinant nuclear receptor LXR- α 3 polypeptides is desired and a eukaryotic host is contemplated, it is most desirable to employ a vector such as a plasmid, that incorporates a eukaryotic origin of replication. Additionally, for the purposes of expression in eukaryotic systems, one desires to position the nuclear receptor LXR- α 3 encoding sequence or if desired parts thereof (SEQ ID NO. 1) adjacent to and under the control of an effective eukaryotic promoter. To bring a coding sequence under control of a promoter, whether it is eukaryotic or prokaryotic, what is generally needed is to position the 5' end of the translation initiation site of the proper translational reading frame of the polypeptide between about 1 and about 2000 nucleotides 3' of or downstream with respect to the promoter chosen.

[0073] Furthermore, where eukaryotic expression is anticipated, one would typically desire to incorporate into the transcriptional unit which includes the nuclear receptor LXR- α 3 polypeptide, an appropriate polyadenylation site.

[0074] The invention provides homogeneous compositions of mammalian nuclear receptor LXR- α 3 polypeptide produced by transformed prokaryotic or eukaryotic cells as provided herein. Such homogeneous compositions are intended to be comprised of mammalian nuclear receptor LXR- α 3 protein that comprises at least 90% of the protein in such homogeneous composition. The invention also provides membrane preparation from cells expressing mammalian nuclear receptor LXR- α 3 polypeptide as the result of transformation with a recombinant expression construct, as described here.

[0075] Within the scope of the present invention the terms recombinant protein or coding sequence both also include tagged versions of the protein depicted in SEQ ID NO. 2, and/or encoded by the nucleic acids according to the invention and fusion proteins of said proteins or parts thereof such as splice variants with any other recombinant protein. Tagged versions here means that small epitopes of 3-20 amino acids are added to the original protein by extending the coding sequence either at the 5' or the 3' terminus leading to N-terminal or C-terminal extended proteins respectively, or that such small epitopes are included elsewhere in the protein. The same applies for fusion proteins where the added sequences are coding for longer proteins, varying between 2 and 100 kDa. Tags and fusion proteins are usually used to facilitate purification of recombinant proteins by specific antibodies or affinity matrices or to increase solubility of recombinant proteins within the expression host. Fusion proteins are also of major use as essential parts of yeast two hybrid screens for interaction partners of recombinant proteins.

[0076] Tags used in the scope of the present invention may include but are not limited to the following: EEF (alpha Tubulin), B-tag (QYPALT), E tag (GAPVPYPDPLEPR) c-myc Tag (EQKLISEEDL), Flag epitope (DYKDDDDK, HA tag (YPYDVPDYA), 6 or 10 x His Tag, HSV (QPELAPEDPED), Pk-Tag (GKIPNPLLGLDST), protein C (EDQVD-PRLIDGK), T7 (MASMTGGQQMG), VSV-G (YTDIEMNRLGK), Fusion proteins may include Thioredoxin, Glutathion-transferase (GST), Maltose binding Protein (MBP), Cellulose Binding protein (CBD), chitin binding protein, ubiquitin, the Fc part of Immunoglobulins, and the IgG binding domain of *Staphylococcus aureus* protein A. These examples of course are illustrative and not limiting and the standard amino acid one letter code was used above.

[0077] For expression of recombinant proteins in living cells or organisms, vector constructs harboring recombinant LXR- α 3 nuclear receptor as set forth in SEQ ID NO. 1 are transformed or transfected into appropriate host cells. Preferably, a recombinant host cell of the present invention is transfected with a polynucleotide of SEQ ID NO. 1.

[0078] Means of transforming or transfecting cells with exogenous polynucleotide such as DNA molecules are well known in the art and include techniques such as calcium-phosphate- or DEAE-dextran-mediated transfection, protoplast fusion, electroporation, liposome mediated transfection, direct microinjection and virus infection (Sambrook et al., 1989).

[0079] The most frequently applied technique for transformation of prokaryotic cells is transformation of bacterial cells after treatment with calciumchloride to increase permeability (Dagert & Ehrlich, 1979), but a variety of other methods is also available for one skilled in the art.

[0080] The most widely used method for transfection of eukaryotic cells is transfection mediated by either calcium phosphate or DEAE-dextran. Although the mechanism remains obscure, it is believed that the transfected DNA enters the cytoplasm of the cell by endocytosis and is transported to the nucleus. Depending on the cell type, up to 90% of a population of cultured cells may be transfected at any one time. Because of its high efficiency, transfection mediated by calcium phosphate or DEAE-dextran is the method of choice for studies requiring transient expression of the foreign nucleic acid in large numbers of cells. Calcium phosphate-mediated transfection is also used to establish cell lines that integrate copies of the foreign DNA, which are usually arranged in head-to-tail tandem arrays into the host cell genome.

[0081] In the protoplast fusion method, protoplasts derived from bacteria carrying high numbers of copies of a plasmid of interest are mixed directly with cultured mammalian cells. After fusion of the cell membranes (usually with polyethylene glycol), the contents of the bacterium are delivered into the cytoplasm of the mammalian cells and the plasmid DNA is transported to the nucleus. Protoplast fusion is not as efficient as transfection for many of the cell lines that are commonly used for transient expression assays, but it is useful for cell lines in which endocytosis of DNA occurs inefficiently. Protoplast fusion frequently yields multiple copies of the plasmid DNA tandemly integrated into the host chromosome.

[0082] The application of brief, high-voltage electric pulses to a variety of mammalian and plant cells leads to the formation of nanometer sized pores in the plasma membrane. DNA is taken directly into the cell cytoplasm either through these pores or as a consequence of the redistribution of membrane components that accompanies closure of the pores. Electroporation may be extremely efficient and may be used both for transient expression of cloned genes and for establishment of cell lines that carry integrated copies of the gene of interest. Electroporation, in contrast to calcium phosphate-mediated transfection and protoplast fusion, frequently gives rise to cell lines that carry one, or at most a few, integrated copies of the foreign DNA.

[0083] Liposome transfection involves encapsulation of DNA and RNA within liposomes, followed by fusion of the liposomes with the cell membrane. The mechanism of how DNA is delivered into the cell is unclear but transfection efficiencies may be as high as 90%.

[0084] Direct microinjection of a DNA molecule into nuclei has the advantage of not exposing DNA to cellular compartments such as low-pH endosomes. Microinjection is therefore used primarily as a method to establish lines of cells that carry integrated copies of the DNA of interest.

[0085] The use of adenovirus as a vector for cell transfection is well known in the art. Adenovirus vector-mediated cell transfection has been reported for various cells (Stratford-Perricaudet et al., 1992).

[0086] A transfected cell may be prokaryotic or eukaryotic, transfection may be transient or stable. Where it is of interest to produce a full length human or mouse LXR- α 3 protein, cultured mammalian mouse, or human cells are of particular interest.

[0087] In another aspect, the recombinant host cells of the present invention are prokaryotic host cells. In addition to prokaryotes, eukaryotic microbes, such as yeast may also be used illustrative examples for suitable cells and organisms for expression of recombinant proteins are belonging to but not limited to the following examples: Insect cells, such as *Drosophila* Sf21, SF9 cells or others, Expression strains of *Escherichia coli*, such as XL1 blue, BRL21, M15, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Hansenula polymorpha* and *Pichia pastoris* strains, immortalised mammalian cell lines such as AIT-20, VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and W138, BHK, COSM6, COS-7, 293 and MDCK cells, BHK-21 cells, Att 20HeLa cells, HeK 294, T47 D cells and others.

[0088] Expression of recombinant proteins within the scope of this invention can also be performed *in vitro*. This may occur by a two step procedure, thereby producing first mRNA by *in vitro* transcription of an apt polynucleotide construct

followed by in vitro translation with convenient cellular extracts. These cellular extracts may be reticulocyte lysates but are not limited to this type. In vitro transcription may be performed by T7 or SP6 DNA polymerase or any other RNA polymerase which can recognise *per se* or with the help of accessory factors the promoter sequence contained in the recombinant DNA construct of choice. Alternatively one of the recently made available one step coupled transcription/translation systems may be used for in vitro translation of DNA coding for the proteins of this invention, e.g. from Roche Molecular Biochemicals. One illustrative but not limiting example for such a system is the TNT® T7 Quick System by Promega.

[0089] Expression of recombinant proteins in transfected cell may occur constitutively or upon induction. Procedures depend on the Cell/vector combination used and are well known in the art. In all cases, transfected cells are maintained for a period of time sufficient for expression of the recombinant LXR- α 3 nuclear receptor protein. A suitable maintenance time depends strongly on the cell type and organism used and is easily ascertainable by one skilled in the art. Typically, maintenance time is from about 2 hours to about 14 days. For the same reasons and for sake of protein stability and solubility incubation temperatures during maintenance time may vary from 20°C to 42 °C.

[0090] Recombinant proteins are recovered or collected either from the transfected cells or the medium in which those cells are cultured. Recovery comprises cell disruption, isolation and purification of the recombinant protein. Isolation and purification techniques for polypeptides are well-known in the art and include such procedures as precipitation, filtration, chromatography, electrophoresis and the like.

[0091] In a preferred embodiment, purification includes but is not limited to affinity purification of tagged or non-tagged recombinant proteins. This is a well established robust technique easily adapted to any tagged protein by one skilled in the art. For affinity purification of tagged proteins, small molecules such as glutathione, maltose or chitin, specific proteins such as the IgG binding domain of Staphylococcus aureus protein A, antibodies or specific chelates which bind with high affinity to the tag of the recombinant protein are employed. For affinity purification of non-tagged proteins specific monoclonal or polyclonal antibodies, which were raised against said protein, can be used. Alternatively immobilised specific interactors of said protein may be employed for affinity purification. Interactors include native or recombinant proteins as well as native or artificial specific low molecular weight ligands.

CHEMICAL SYNTHESIS OF THE POLYPEPTIDE ACCORDING TO THE INVENTION:

[0092] Alternatively, the protein itself may be produced using chemical methods to synthesise any of the amino acid sequences according to the invention or that is encoded by the nucleotide sequence according to the invention (SEQ ID NO. 1) and/or a portion thereof and/or splice variants thereof. For example, peptide synthesis can be performed using conventional Merrifield solid phase f-Moc or t-Boc chemistry or various solid-phase techniques (Roberge, J. Y. et al. (1995) Science 269: 202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer). The newly synthesised peptide(s) may be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, T. (1983) Proteins, Structures and Molecular Principles, WH Freeman and Co., New York, N.Y.). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; Creighton, supra). Additionally, the amino acid sequence according to the invention, i.e. SEQ ID NO. 2 or the sequence that is encoded by SEQ ID NO. 1 or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

SCREENING ASSAYS

[0093] The invention also concerns a method for screening for agents which are capable of inhibiting the cellular function of the nuclear receptor LXR- α 3 comprising the steps of contacting one or more candidate agents with a polypeptide according to the invention, removing unbound agent(s) and detecting whether the agent(s) interact with the polypeptide of the nuclear receptor.

[0094] The invention also concerns method for inhibiting the cellular function of the nuclear receptor LXR- α 3, comprising the steps of contacting a cell with a binding agent of a polypeptide previously identified as outlined herein whereby the cellular function of LXR- α 3 is inhibited.

[0095] Such a binding agent may be an antibody, RNA, an anti-sense oligonucleotide, a ribozyme or one of substances shown below or identified in a respective assay as disclosed herein.

[0096] In still a further embodiment, the present invention concerns a method for identifying new nuclear receptor inhibitory or stimulatory substances, which may be termed as "candidate substances". It is contemplated that this screening technique proves useful in the general identification of compounds that serve the purpose of inhibiting or stimulating nuclear receptor activity.

[0097] In one embodiment of the invention the following substances are disclosed as potential interactors of the nuclear receptor according to the invention:

[0098] Steroids: 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 24(S),25 epoxcholesterol, T-0314407 (Tularik, Schultz et al., Gen Dev 14, 2831-2838 (2000)), T-0901317 (Tularik, Schultz et al., Gen Dev 14, 2831-2838 (2000)), 3alpha-6alpha-dihydroxy-5beta-cholanoic acid-N-methyl-N-methoxy-24-amide (Hypocholeamide, Song C, et al., Steroids. 65 423-427 (2000)), cholesterol sulfates,

5 **[0099]** Other substances: arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid and other polyunsaturated fatty acids (Yoshikawa et al., J Biol Chem 277,1705-1711 (2002)), geranyl-geranyl-pyrophosphate Gan X, et al., J Biol Chem. 276 48702-48708 (2001), acetylpodocarpic dimer.

[0100] This also includes the use of heteromultimeric complexes of the nuclear receptor with other proteins, such as heterodimeric complexes with RXR, or any other binding partner.

10 **[0101]** Accordingly, in screening assays to identify pharmaceuticals agents which affect nuclear receptor activity, it is proposed that compounds isolated from natural sources, such as fungal extracts, plant extracts, bacterial extracts, higher eukaryotic cell extracts, or even extracts from animal sources, or marine, forest or soil samples, may be assayed for the presence of potentially useful pharmaceutical agents.

15 **[0102]** It will be understood that that the pharmaceutical agents to be screened could also be derived from chemical compositions or man-made compounds. The candidate substances can also include monoclonal or polyclonal antibodies, peptides or proteins, such as those derived from recombinant DNA technology or by other means, including chemical peptide synthesis. The active compounds may include fragments or parts or derivatives of naturally-occurring compounds or may be only found as active combinations of known compounds which are otherwise inactive. We anticipate that such screens will in some cases lead to the isolation of agonists of nuclear receptors, in other cases to the isolation of antagonists. In other instances, substances will be identified that have mixed agonistic and antagonistic effects, or affect nuclear receptors in any other way.

CELL BASED ASSAYS

25 **[0103]** To identify a candidate substance capable of influencing LXR- α 3 nuclear receptor activity, one first obtains a recombinant cell line. One designs the cell line in such a way that the activity of the nuclear receptor leads to the expression of a protein which has an easily detectable phenotype (a reporter), such as luciferase, fluorescent proteins such as green or red fluorescent protein, beta-galactosidase, alpha-galactosidase, beta-lactamase, chloramphenicol-acetyl-transferase, beta-glucuronidase, or any protein which can be detected by a secondary reagent such as an antibody.

30 **[0104]** Methods for detecting proteins using antibodies, such as ELISA assays, are well known to those skilled in the art.

35 **[0105]** Here, the amount of reporter protein present reflects the transcriptional activity of the nuclear receptor. This recombinant cell line is then screened for the effect of substances on the expression of the reporters, thus measuring the effect of these substances on the activity of the nuclear receptor. These substances can be derived from natural sources, such as fungal extracts, plant extracts, bacterial extracts, higher eukaryotic cell extracts, or even extracts from animal sources, or marine, forest or soil samples, may be assayed for the presence of potentially useful pharmaceutical agents. It will be understood that that the pharmaceutical agents to be screened may be derived from chemical compositions or man-made compounds.

40 **[0106]** The candidate substances can also include monoclonal or polyclonal antibodies, peptides or proteins, such as those derived from recombinant DNA technology or by other means, including chemical peptide synthesis. The active compounds may include fragments or parts or derivatives of naturally-occurring compounds or may be only found as active combinations of known compounds which are otherwise inactive.

45 **[0107]** In general the assay comprises, contacting a suitable cell containing a reporter under the control of the LXR- α 3 nuclear receptor with a test compound, monitoring said host cell for the expression of the reporter gene, wherein expression of the reporter reflects the transcriptional activity of the nuclear receptor LXR- α 3, and therefore reflects effects of the compound on the nuclear receptor.

50 **[0108]** In other embodiments of the invention assays are included where measuring the activity of a dimer of the nuclear receptor LXR- α 3 and another protein, such as RXR takes place. Further included are assays aiming at the identification of compounds which specifically influence only the monomeric, homodimeric or homomultimeric form of the nuclear receptor, or influencing only multimeric forms of the nuclear receptor. Such assays include measuring the effect of a compound on the nuclear receptor in the absence of a binding partner, and measuring the effect of a compound on the nuclear receptor in the presence of a binding partner, such as RXR. One skilled in the art will find numerous more assays which are equally covered by the invention.

55 **[0109]** A cell line where the activity of a nuclear receptor determines the expression of a reporter can be obtained by creating a fusion gene driving the expression of a fusion protein consisting of the ligand-binding domain of the LXR- α 3 nuclear receptor fused to the DNA binding domain of a transcription factor with a known specificity for a given DNA sequence (the binding site). This DNA sequence (the binding site) can then be inserted in one or multiple copies before

(5') to the promoter driving the expression of the reporter. Transcription factors useful for this approach include bacterial proteins, such as *lexA*, yeast proteins, such as Gal4, mammalian proteins such as NFkappaB or NFAT, the glucocorticoid receptor, the estrogen receptor, or plant proteins. The binding sites for these proteins can all be used in combination with the appropriate transcription factor to generate a useful reporter assay.

[0110] Another way to screen for inhibitors is to identify binding sites on DNA for the LXR- α 3 nuclear receptor, and operatively link this binding site to a promoter operatively linked to a reporter gene. Included among others are binding sites for heterodimers of the LXR- α 3 nuclear receptor with another protein, such as RXR.

[0111] Furthermore, transgenic animals described in the invention can be used to derive cell lines useful for cellular screening assays.

[0112] Cell lines useful for such an assay include many different kinds of cells, including prokaryotic, animal, fungal, plant and human cells. Yeast cells can be used in this assay, including *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* cells.

[0113] Another way to build cellular assays to measure the effect of compounds is the use of the yeast two hybrid system (see for example see, for example, U.S. Pat. No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Biotechniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; PCT Publication No. WO 94/10300, and U.S. Pat. No. 5,667,973), and or possible variants of the basic two hybrid system as discussed e.g. in Vidal M, Legrain P, Nucleic Acids Res. 1999 Feb 15;27(4):919-29. Briefly, the two hybrid assay relies on reconstituting in vivo a functional transcriptional activator protein from two separate fusion proteins. In particular, the method makes use of chimeric genes which express hybrid proteins. To illustrate, a first hybrid gene comprises the coding sequence for a DNA-binding domain of a transcriptional activator fused in frame to the coding sequence for a TI polypeptide. The second hybrid protein encodes a transcriptional activation domain fused in frame to a sample gene from a cDNA library. If the bait and sample hybrid proteins are able to interact, e.g., form a TI-dependent complex, they bring into close proximity the two domains of the transcriptional activator. This proximity is sufficient to cause transcription of a reporter gene which is operably linked to a transcriptional regulatory site responsive to the transcriptional activator, and expression of the reporter gene can be detected and used to score for the interaction of the TI and sample proteins.

[0114] In such assays, one primarily measures the effect of a compound on a given interaction involving the LXR- α 3 nuclear receptor and a binding protein. In a preferred embodiment of the invention systems using other hosts such as prokaryotes as *E. coli*, or eukaryotic mammalian cells are described.

[0115] Two hybrid systems using hybrid protein fusions with other proteins than transcription factors, including enzymes such as beta-galactosidase or *dihydrofolate reductase* may also be applied. These assays are useful both to monitor the effect of a compound, including peptides, proteins or nucleic acids on an interaction of a nuclear receptor with a given binding partner, as well as to identify novel proteins or nucleic acids interacting with the nuclear receptor.

[0116] Monitoring the influence of compounds on cells may be applied not only in basic drug screening, but also in clinical trials. In such clinical trials, the expression of a panel of genes may be used as a "read out" of a particular drug's therapeutic effect.

CELL-FREE ASSAYS

[0117] Recombinant forms of the polypeptide according to SEQ ID NO. 2 or as encoded by the nucleic acids according to the invention can be used in cell-free screening assays aiming at the isolation of compounds affecting the activity of nuclear receptors. In such an assay, the nuclear receptor polypeptide is brought into contact with a substance to test if the substance has an effect on the activity of the LXR- α 3 receptor.

[0118] The detection of an interaction between an agent and a receptor may be accomplished through techniques well-known in the art. These techniques include but are not limited to centrifugation, chromatography, electrophoresis and spectroscopy. The use of isotopically labeled reagents in conjunction with these techniques or alone is also contemplated. Commonly used radioactive isotopes include ^3H , ^{14}C , ^{22}Na , ^{32}P , ^{33}P , ^{35}S , ^{45}Ca , ^{60}Co , ^{125}I , and ^{131}I . Commonly used stable isotopes include ^2H , ^{13}C , ^{15}N , ^{18}O .

[0119] For example, if an agent binds to the receptor of the present invention, the binding may be detected by using radiolabeled agent or radiolabeled receptor. Briefly, if radiolabeled agent or radiolabeled receptor is utilized, the agent-receptor complex may be detected by liquid scintillation or by exposure to x-ray film or phosphor-imaging devices.

[0120] One way to screen for substances affecting nuclear receptor activity is to measure the effect of the binding of nuclear receptors to ligands, such as cofactors, activators, repressors, DNA, RNA, proteins, antibodies, peptides or other substances, including chemical compounds known to affect receptor activity. Assays measuring the binding of a protein to a ligand are well known in the art, such as ELISA assays, FRET assays, bandshift assays, plasmon-resonance based assays, scintillation proximity assays, fluorescence polarization assays.

[0121] In one example, a mixture containing the LXR- α 3 polypeptide, effector and candidate substance is allowed to incubate. The unbound effector is separable from any effector/receptor complex so formed. One then simply meas-

ures the amount of each (e.g., versus a control to which no candidate substance has been added). This measurement may be made at various time points where velocity data is desired. From this, one determines the ability of the candidate substance to alter or modify the function of the receptor.

[0122] Numerous techniques are known for separating the effector from effector/receptor complex, and all such methods are intended to fall within the scope of the invention. This includes the use of thin layer chromatographic methods (TLC), HPLC, spectrophotometric, gas chromatographic/mass spectrophotometric or NMR analyses. Another method of separation is to immobilise one of the binding partners on a solid support, and to wash away any unbound material. It is contemplated that any such technique may be employed so long as it is capable of differentiating between the effector and complex, and may be used to determine enzymatic function such as by identifying or quantifying the substrate and product.

[0123] A screening assay provides a LXR- α 3 receptor under conditions suitable for the binding of an agent to the LXR- α 3 receptor. These conditions include but are not limited to pH, temperature, tonicity, the presence of relevant cofactors, and relevant modifications to the polypeptide such as glycosylation or lipidation. It is contemplated that the receptor can be expressed and utilised in a prokaryotic or eukaryotic cell. The host cell expressing the LXR- α 3 receptor can be used whole or the receptor can be isolated from the host cell. The LXR- α 3 receptor can be membrane bound in the membrane of the host cell or it can be free in the cytosol of the host cell. The host cell can also be fractionated into sub-cellular fractions where the receptor can be found. For example, cells expressing the receptor can be fractionated into the nuclei, the endoplasmic reticulum, vesicles, or the membrane surfaces of the cell.

[0124] pH is preferably from about a value of 6.0 to a value of about 8.0, more preferably from about a value of about 6.8 to a value of about 7.8, and most preferably, about 7.4. In a preferred embodiment, temperature is from about 20°C degrees to about 50°C degrees more preferably, from about 30°C degrees to about 40°C degrees and even more preferably about 37°C degrees. Osmolality is preferably from about 5 milliosmols per liter (mosm/L) to about 400 mosm/L, and more preferably, from about 200 milliosmols per liter to about 400 mosm/L and, even more preferably from about 290 mosm/L to about 310 mosm/L. The presence of cofactors can be required for the proper functioning of the LXR- α 3 receptor. Typical cofactors include sodium, potassium, calcium, magnesium, and chloride. In addition, small, non-peptide molecules, known as prosthetic groups may also be required. Other biological conditions needed for receptor function are well-known in the art.

[0125] It is well-known in the art that proteins can be reconstituted in artificial membranes, vesicles or liposomes. (Danboldt et al., 1990). The present invention contemplates that the receptor can be incorporated into artificial membranes, vesicles or liposomes. The reconstituted receptor can be utilised in screening assays.

[0126] It is further contemplated that a receptor of the present invention can be coupled to a solid support, e.g., to agarose beads, polyacrylamide beads, polyacrylic, sepharose beads or other solid matrices capable of being coupled to polypeptides. Well-known coupling agents include cyanogen bromide (CNBr), carbonyldiimidazole, tosyl chloride, diaminopimelimidate, and glutaraldehyde.

[0127] In a typical screening assay for identifying candidate substances, one employs the same recombinant expression host as the starting source for obtaining the receptor polypeptide, generally prepared in the form of a crude homogenate. Recombinant cells expressing the receptor are washed and homogenised to prepare a crude polypeptide homogenate in a desirable buffer such as disclosed herein. In a typical assay, an amount of polypeptide from the cell homogenate, is placed into a small volume of an appropriate assay buffer at an appropriate pH. Candidate substances, such as agonists and antagonists, are added to the admixture in convenient concentrations and the interaction between the candidate substance and the receptor polypeptide is monitored.

[0128] Where one uses an appropriate known substrate for the LXR- α 3 receptor, one can, in the foregoing manner, obtain a baseline activity for the recombinantly produced LXR- α 3 receptor. Then, to test for inhibitors or modifiers of the receptor function, one can incorporate into the admixture a candidate substance whose effect on the LXR- α 3 receptor is unknown. By comparing reactions which are carried out in the presence or absence of the candidate substance, one can then obtain information regarding the effect of the candidate substance on the normal function of the receptor.

[0129] Accordingly, this aspect of the present invention will provide those of skill in the art with methodology that allows for the identification of candidate substances having the ability to modify the action of nuclear receptor polypeptides in one or more manners.

[0130] Additionally, screening assays for the testing of candidate substances are designed to allow the determination of structure-activity relationships of agonists or antagonists with the receptors, e.g., comparisons of binding between naturally-occurring hormones or other substances capable of interacting with or otherwise modulating the receptor; or comparison of the activity caused by the binding of such molecules to the receptor. Such assays involve FRET (fluorescence resonance energy transfer) assays, ALPHA-screen, and/or BRET (bioluminescence resonance energy transfer) assays.

[0131] In certain aspects, the polypeptides of the invention are crystallised in order to carry out x-ray crystallographic studies as a means of evaluating interactions with candidate substances or other molecules with the nuclear receptor

polypeptide. For instance, the purified recombinant polypeptides of the invention, when crystallised in a suitable form, are amenable to detection of intra-molecular interactions by x-ray crystallography. In another aspect, the structure of the polypeptides can be determined using nuclear magnetic resonance.

5 PHARMACEUTICAL COMPOSITION:

[0132] This invention provides a pharmaceutical composition comprising an effective amount of a agonist or antagonist drug identified by the method described herein and a pharmaceutically acceptable carrier. Such drugs and carrier can be administered by various routes, for example oral, subcutaneous, intramuscular, intravenous or intracerebral.

10 **[0133]** This invention provides a method of treating metabolic disorders, immunological indications, hormonal dysfunctions, neurosystemic diseases wherein the abnormality is improved by reducing the activity of LXR- α 3 receptor or blocking the binding of ligands to a LXR- α 3 receptor, which method comprises administering an effective amount of the antagonist-containing pharmaceutical composition described above to suppress the subject's appetite. Similarly, 15 the invention also provides methods for treating diseases and conditions resulting from metabolic disorders, immunological indications, hormonal dysfunctions, neurosystemic diseases, which method comprises administering an effective amount of an agonist-containing pharmaceutical composition described above.

20 TRANSFORMATION OF CELLS AND DRUG SCREENING:

[0134] The recombinant expression constructs of the present invention are useful in molecular biology to transform cells which do not ordinarily express LXR- α 3 to thereafter express this receptor.

[0135] Such cells are useful as intermediates for making cellular preparations useful for receptor binding assays, which are in turn useful for drug screening. Drugs identified from such receptor assays can be used for the treatment 25 of metabolic disorders, immunological indications, hormonal dysfunctions, and/or neurosystemic diseases.

[0136] The recombinant expression constructs of the present invention are also useful in gene therapy. Cloned genes of the present invention, or fragments thereof, may also be used in gene therapy carried out by homologous recombination or site-directed mutagenesis. See generally Thomas & Capecchi, Cell 51, 503-512 (1987); Bertling, Bioscience Reports 7, 107-112 (1987); Smithies et al., Nature 317, 230-234 (1985).

30 **[0137]** Oligonucleotides of the present invention are useful as diagnostic tools for probing LXR- α 3 expression in tissues. For example, tissues are probed *in situ* with oligonucleotide probes carrying detectable groups by conventional autoradiographic techniques, as explained in greater detail in the Examples below, to investigate native expression of this receptor or pathological conditions relating thereto. Further, chromosomes can be probed to investigate the presence or absence of the LXR- α 3, and potential pathological conditions related thereto, as also illustrated by the Exam- 35 ples below. Probes according to the invention should generally be at least about 15 nucleotides in length to prevent binding to random sequences, but, under the appropriate circumstances may be smaller (see above for details on hybridisation).

40 ANTIBODIES AGAINST THE LXR- α 3 NUCLEAR RECEPTOR PROTEIN OR POLYPEPTIDE

[0138] Another aspect of the invention includes an antibody specifically reactive with the protein or any part of the protein according to the invention (SEQ ID NO. 2) and or a polypeptide encoded by the nucleotide sequence of the nuclear receptor LXR- α 3 (The term "antibody" refers to intact molecules as well as fragments thereof, such as Fa, F 45 (ab).sub.2, and Fv, which are capable of binding the epitopic determinant.) By using immunogens derived from the polypeptide according to the invention (SEQ ID NO. 2) and/or encoded by the nucleic acids according to the invention, anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (E. Howell & D. Lane. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory (1988)).

[0139] A polyclonal antibody is prepared by immunising a mammal, such as a mouse, a hamster or rabbit with an immunogenic form of the polypeptide, i.e. the human LXR- α 3 polypeptide of the present invention, and collecting 50 antisera from that immunised animal. Because of the relatively large blood volume of rabbits, a rabbit is a preferred choice for production of polyclonal antibodies.

[0140] As an immunising antigen, fusion proteins, intact polypeptides or fragments containing small peptides of interest can be used. They can be derived by expression from a cDNA transfected in a host cell with subsequent recovering of the protein/peptide or peptides can be synthesised chemically (e.g. oligopeptides with 10-15 residues in length). 55 Important tools for monitoring the function of the gene according to the present invention, i.e. encoded by a sequence according to SEQ ID NO. 1 (or portions thereof or splice variants thereof) are antibodies against various domains of the protein according to the invention. Various oligopeptides from the N- and C-terminal sequences and the DBD/hinge region of the protein can be used as antigens.

[0141] A given polypeptide or polynucleotide may vary in its immunogenicity. It is often necessary to couple the immunogen (e.g. the polypeptide) with a carrier. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunise the animal in the presence of an adjuvant, a non-specific stimulator of the immune response in order to enhance immunogenicity. The production of polyclonal antibodies is monitored by detection of antibody titers in plasma or serum at various time points following immunisation. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies. When a desired level of immunogenicity is obtained, the immunised animal may be bled and the serum isolated, stored and purified.

[0142] To produce monoclonal antibodies, antibody-producing cells (e.g. spleen cells) from an immunised animal (preferably mouse or rat) are fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Where the immunized animal is a mouse, a preferred myeloma cell is the murine NS-1 myeloma cell. Such techniques are well known in the art, and include, for example, the hybridoma technique (originally developed by Kohler & Milstein. *Nature* 256: 495-497 (1975)), the human B cell hybridoma technique (Kozbar *et al. Immunology Today* 4:72 (1983)), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al. Monoclonal Antibodies and Cancer Therapy. Alan R. Liss, Inc. pp. 77-96 (1985)).*

[0143] The fused spleen/myeloma cells are cultured in a selective medium to select fused spleen/myeloma cells from the parental cells. Fused cells are separated from the mixture of non-fused parental cells, for example, by the addition of agents that block the *de novo* synthesis of nucleotides in the tissue culture media. This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants for reactivity with an antigen-polypeptide. The selected clones may then be propagated indefinitely to provide the monoclonal antibody in convenient quantity.

[0144] The creation of antibodies which specifically bind the polypeptide according to the invention (SEQ ID NO. 2) and/or encoded by the nucleotide sequence of the nuclear receptor LXR- α 3 or its complement (SEQ ID NO. 1) provides an important utility in immunolocalization studies, and may play an important role in the diagnosis and treatment of receptor disorders. The antibodies may be employed to identify tissues, organs, and cells which express or the nuclear receptor LXR- α 3. Antibodies can be used diagnostically in immuno-precipitation and immuno-blotting to detect and evaluate nuclear receptor LXR- α 3 protein levels in tissue or from cells in bodily fluid as part of a clinical testing procedure.

[0145] Monoclonal antibodies provided by the present invention are also produced by recombinant genetic methods well known to those of skill in the art, and the present invention encompasses antibodies made by such methods that are immunologically reactive with an epitope of a mammalian nuclear LXR- α 3 receptor protein or peptide.

[0146] The present invention encompasses fragments of the antibody that are immunologically reactive with an epitope of a mammalian nuclear LXR- α 3 receptor protein or peptide. Such fragments are produced by any number of methods, including but not limited to proteolytic cleavage, chemical synthesis or preparation of such fragments by means of genetic engineering technology. The present invention also encompasses single-chain antibodies that are immunologically reactive with an epitope of a mammalian nuclear LXR- α 3 receptor protein or peptide made by methods known to those of skilled in the art.

CHIMERIC ANTIBODIES AND OTHER TYPES OF ANTIBODIES:

[0147] The invention also includes chimeric antibodies, comprised of light chain and heavy chain peptides immunologically reactive to an epitope that is a mammalian nuclear LXR- α 3 receptor protein or peptide. The chimeric antibodies embodied in the present invention include those that are derived from naturally occurring antibodies as well as chimeric antibodies made by means of genetic engineering technology well known to those of skill in the art.

[0148] Also included are methods for the generation of antibodies against LXR- α 3 which rely on the use of phage display systems and related systems, such as described in Hoogenboom HR, de Bruine AP, Hufton SE, Hoet RM, Arends JW, Roovers RC, *Immunotechnology* 1998 Jun;4(1): 1-20, and references therein.

EPITOPES OF THE LXR- α 3 NUCLEAR RECEPTOR

[0149] The present invention also encompasses an epitope of a mammalian nuclear LXR- α 3 receptor protein or peptide that is comprised of sequences and/or a conformation of sequences present in the mammalian nuclear LXR- α 3 receptor protein or peptide molecule. This epitope may be naturally occurring, or may be the result of proteolytic cleavage of the mammalian nuclear LXR- α 3 receptor protein or peptide molecule and isolation of an epitope-containing peptide or may be obtained by synthesis of an epitope-containing peptide using method of genetic engineering technology and synthesised by genetically engineered prokaryotic or eukaryotic cells.

ANTISENSE OLIGONUCLEOTIDES AGAINST LXR- α 3

[0150] Antisense oligonucleotides are short single stranded DNA or RNA molecules which may be used to block the availability of the LXR- α 3 receptor messenger. Synthetic derivatives of ribonucleotides or deoxyribonucleotides and/or PNAs (see above) are equally possible.

[0151] The sequence of an antisense oligonucleotide is at least partially complementary to the sequence (or the gene) of interest. The complementarity of the sequence is in any case high enough to enable the antisense oligonucleotide to bind to the nucleic acid according to the invention or parts thereof. Many examples exist in which the binding of oligonucleotides to the target sequence interfere with the biological function of the targeted sequence (Brysch W, Schlingensiepen KH, Design and application of antisense oligonucleotides in cell culture, in vivo, and as therapeutic agents, Cell Mol Neurobiol 1994 Oct;14(5):557-68; Wagner RW, Gene inhibition using antisense oligodeoxynucleotides, Nature 1994 Nov 24;372(6504):333-5 or Brysch W, Magal E, Louis JC, Kunst M, Klinger I, Schlingensiepen R, Schlingensiepen KH Inhibition of p185c-erbB-2 proto-oncogene expression by antisense oligodeoxynucleotides down-regulates p185-associated tyrosine-kinase activity and strongly inhibits mammary tumor-cell proliferation, Cancer Gene Ther 1994 Jun;1(2):99-105 or Monia BP, Johnston JF, Ecker DJ, Zounes MA, Lima WF, Freier SM Selective inhibition of mutant Ha-ras mRNA expression by antisense oligonucleotides, J Biol Chem 1992 Oct 5;267(28):19954-62 or Bertram J, Palfner K, Killian M, Brysch W, Schlingensiepen KH, Hiddemann W, Kneba M, Reversal of multiple drug resistance in vitro by phosphorothioate oligonucleotides and ribozymes, Anticancer Drugs 1995 Feb;6(1):124-34)

[0152] This interference occurs in most instances at the level of translation, *i.e.* through the inhibition of the translational machinery by oligonucleotides that bind to mRNA, however, two other mechanisms of interference with a given gene's function by oligonucleotides can also be envisioned, (i) the functional interference with the transcription of a gene through formation of a triple helix at the level of genomic DNA and the interference of oligonucleotides with the function of RNA molecules that are executing at least part of their biological function in the untranslated form (Kochetkova M, Shannon MF, Triplex-forming oligonucleotides and their use in the analysis of gene transcription. Methods Mol Biol 2000;130:189-201 Rainer B. Lanzl, Neil J. McKenall, Sergio A. Onatel, Urs Albrecht2, Jiemin Wongl, Sophia Y. Tsail, Ming-Jer Tsail, and Bert W. O'Malley A Steroid Receptor Coactivator, SRA, Functions as an RNA and Is Present in an SRC-1 Complex Cell, Vol. 97, 17-27, April, 1999).

[0153] Antisense oligonucleotides can be conjugated to different other molecules in order to deliver them to the cell or tissue expressing LXR- α 3. For instance the antisense oligonucleotide can be conjugated to a carrier protein (*e.g.* ferritin) in order to direct the oligonucleotide towards the desired target tissue, *i.e.* in case of ferritin predominantly to the liver.

[0154] Antisense expression constructs are expression vector systems that allow the expression-either inducible or uninducible - of a complementary sequence to the LXR- α 3 sequences according to the invention. The potential possibility of such an approach has been demonstrated in many different model systems (von Ruden T, Gilboa E, Inhibition of human T-cell leukemia virus type I replication in primary human T cells that express antisense RNA, J Virol 1989 Feb;63(2):677-82; Nemir M, Bhattacharyya D, Li X, Singh K, Mukherjee AB, Mukherjee BB, Targeted inhibition of osteopontin expression in the mammary gland causes abnormal morphogenesis and lactation deficiency, J Biol Chem 2000 Jan 14;275(2):969-76; Ma L, Gauville C, Berthois Y, Millot G, Johnson GR, Calvo F Antisense expression for amphiregulin suppresses tumorigenicity of a transformed human breast epithelial cell line, Oncogene 1999 Nov 11;18(47):6513-20; Refolo LM, Eckman C, Prada CM, Yager D, Sambamurti K, Mehta N, Hardy J, Younkin SG, Antisense-induced reduction of presenilin 1 expression selectively increases the production of amyloid beta42 in transfected cells, J Neurochem 1999 Dec;73(6):2383-8; Buckley NJ, Abogadie FC, Brown DA, Dayrell M, Caulfield MP, Delmas P, Haley JE, Use of antisense expression plasmids to attenuate G-protein expression in primary neurons, Methods Enzymol 2000;314:136-48).

[0155] According to the invention an antisense expression construct can be constructed with virtually any expression vector capable of fulfilling at least the basic requirements known to those skilled in the art.

[0156] In one embodiment of the invention retroviral expression systems or tissue specific gene expression systems are preferred.

[0157] Current standard technologies for delivering antisense constructs are performed through a conjugation of constructs with liposomes and related, complex-forming compounds, which are transferred via electroporation techniques or via particle-mediated "gene gun" technologies into the cell. Other techniques may be envisioned by one skilled in the art.

[0158] Microinjection still plays a major role in most gene transfer techniques for the generation of germ-line mutants expressing foreign DNA (including antisense RNA constructs) and is preferred embodiment of the present invention.

RIBOZYMES DIRECTED AGAINST LXR- α 3

[0159] Ribozymes are either RNA molecules (Gibson SA, Pellenz C, Hutchison RE, Davey FR, Shillitoe EJ, Induction

of apoptosis in oral cancer cells by an anti-bcl-2 ribozyme delivered by an adenovirus vector, Clin Cancer Res 2000 Jan;6(1):213-22; Folini M, Colella G, Villa R, Lualdi S, Daidone MG, Zaffaroni N, Inhibition of Telomerase Activity by a Hammerhead Ribozyme Targeting the RNA Component of Telomerase in Human Melanoma Cells, J Invest Dermatol 2000 Feb;114(2):259-267; Halatsch ME, Schmidt U, Botefur IC, Holland JF, Ohnuma T, Marked inhibition of glioblastoma target cell tumorigenicity in vitro by retrovirus-mediated transfer of a hairpin ribozyme against deletion-mutant epidermal growth factor receptor messenger RNA, J Neurosurg 2000 Feb;92(2):297-305; Ohmichi T, Kool ET, The virtues of self-binding: high sequence specificity for RNA cleavage by self-processed hammerhead ribozymes, Nucleic Acids Res 2000 Feb 1;28(3):776-783 or DNA molecules (Li J, Zheng W, Kwon AH, Lu Y, In vitro selection and characterization of a highly efficient Zn(II)-dependent RNA-cleaving deoxyribozyme; Nucleic Acids Res 2000 Jan 15;28(2): 481-488) that have catalytic activity. The catalytic activity located in one part of the RNA (or DNA) molecule can be "targeted" to a specific sequence of interest by fusing the enzymatically active RNA molecule sequence with a short stretch of RNA (or DNA) sequence that is complementary to the LXR- α 3 transcript. Such a construct will, when introduced into a cell either physically or via gene transfer of a ribozyme expression construct find the LXR- α 3 sequence (our sequence of interest) and bind via its sequence-specific part to said sequence. The catalytic activity attached to the construct, usually associated with a special nucleic acid structure (people distinguish so called "hammerhead" structures and "hairpin" structures), will then cleave the targeted RNA. The targeted mRNA will be destroyed and cannot be translated efficiently, thus the protein encoded by the mRNA derived from LXR- α 3 will not be expressed or at least will be expressed at significantly reduced amounts.

[0160] In a preferred embodiment the invention covers inducible ribozyme constructs (Koizumi M, Soukup GA, Kerr JN, Breaker RR, Allosteric selection of ribozymes that respond to the second messengers cGMP and cAMP, Nat Struct Biol 1999 Nov;6(11):1062-1071).

[0161] In a further preferred embodiment the invention concerns the use of "bivalent" ribozymes (multimers of catalytically active nucleic acids) as described in (Hamada M, Kuwabara T, Warashina M, Nakayama A, Taira K, Specificity of novel allosterically trans- and cis-activated connected maxizymes that are designed to suppress BCR-ABL expression FEBS Lett 1999 Nov 12;461(1-2):77-85).

TRANSGENIC ANIMALS CARRYING THE LXR- α 3 NUCLEAR RECEPTOR

[0162] Also provided by the present invention are non-human transgenic animals grown from germ cells transformed with the LXR- α 3 nucleic acid sequence according to the invention and that express the LXR- α 3 receptor according to the invention and offspring and descendants thereof. Also provided are transgenic non-human mammals comprising a homologous recombination knockout of the native LXR- α 3 receptor, as well as transgenic non-human mammals grown from germ cells transformed with nucleic acid antisense to the LXR- α 3 nucleic acid of the invention and offspring and descendants thereof. Further included as part of the present invention are transgenic animals which the native LXR- α 3 receptor has been replaced with the human homologue. Of course, offspring and descendants of all of the foregoing transgenic animals are also encompassed by the invention.

[0163] Transgenic animals according to the invention can be made using well known techniques with the nucleic acids disclosed herein. E.g., Leder et al., U.S. Patent Nos. 4,736,866 and 5,175,383; Hogan et al., Manipulating the Mouse Embryo, A Laboratory Manual (Cold Spring Harbor Laboratory (1986)); Capecchi, Science 244, 1288 (1989); Zimmer and Gruss, Nature 338, 150 (1989); Kuhn et al., Science 269, 1427 (1995); Katsuki et al., Science 241, 593 (1988); Hasty et al., Nature 350, 243 (1991); Stacey et al., Mol. Cell Biol. 14, 1009 (1994); Hanks et al., Science 269, 679 (1995); and Marx, Science 269, 636 (1995). Such transgenic animals are useful for screening for and determining the physiological effects of LXR- α 3 receptor agonists and antagonist.

[0164] Consequently, such transgenic animals are useful for developing drugs to regulate physiological activities in which LXR- α 3 participates.

[0165] The following examples are provided for illustrative purposes only and are not intended, nor should they be construed, as limiting the invention in any manner.

MODELLING OF THE STRUCTURE OF LXR- α 3

[0166] The novel nuclear receptor sequences disclosed herein may be used for various in silico, i.e. computer analyses. Such analyses may be for example nuclear receptor specific sequence alignments which permit the identification of domains and even new receptors. The novel domain sequences disclosed herein may be used in order to create domain specific hidden markov models (hmms) or simply as search sequences.

[0167] In a preferred embodiment this similarity search tool is the BLAST algorithm. (Altschul, Stephen F., Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman (1990). Basic local alignment search tool. J. Mol. Biol. 215: 403-10 and the sequence used is one of those disclosed herein.

[0168] Another search tool that may be used is FASTA (W. R. Pearson and D. J. Lipman (1988), "Improved Tools

for Biological Sequence Analysis", PNAS 85:2444- 2448, and W. R. Pearson (1990) "Rapid and Sensitive Sequence Comparison with FASTP and FASTA" Methods in Enzymology 183:63-98).

[0169] The invention is not limited to one particular type of search tool. In one embodiment of the invention search tools are used that do not search by sequence similarity but by applying sequence profiles such as a profile generated when applying the Profile Hidden Markov Model.

[0170] Profile Hidden Markov Models also called "Hidden Markov Models", here abbreviated as HMM, are statistical models representing the consensus of the primary structure of a sequence family. The profiles use scores specific of the position of amino acids (or nucleotides) and position specific scores for the opening or the expansion of an insertion or deletion. Methods for the creation of profiles, starting from multiple alignments, have been introduced by Taylor (1986), Gribskov et al. (1987), Barton (1990) and Heinikoff (1996).

[0171] HMMs provide an utterly probabilistic description of profiles, *i.e.* Bayes' theory rules the positioning of all probability (evaluation) parameters (compare Krogh et al. 1994, Eddy 1996 und Eddy 1998). The central idea behind this is that a HMM is a finite model describing the probability distribution of an infinite number of possible sequences. The HMM consists of a number of states corresponding with the columns of a multiple alignment as it is usually depicted. Each state emits symbols (remainders) corresponding with the probability of the symbol emission (specific of the respective state), and the states are linked with each other by probabilities of the changing of states. Starting from one specific state, a succession of states is generated by changing from one state to the other in accordance with the probability of the changing of states, until a final state has been reached. Each state then emits symbols according to the probability distribution of emissions specific of this state, creating an observable sequence of symbols.

[0172] The attribute "hidden" has been derived from the fact that the underlying sequence of states cannot be observed. Only the sequence of symbols is visible. An assessment of the probabilities of changing of states and of emissions (the training of the model) is achieved by dynamic programming algorithms implemented in the HMMER package.

[0173] The sequences according to the invention may be aligned with other nuclear receptor sequences in order to create a multiple sequence alignment which is used as the basis for the creation of a HMM.

[0174] If an existing HMM and a sequence are given, the probability that the HMM could generate the sequence in question, can be calculated. The HMMER package provides a numerical quantity (the Score) in proportion to this probability, *i.e.* the information content of the sequence indicated as bits, measured according to the HMM.

[0175] See also Barton, G.J. (1990): Protein multiple alignment and flexible pattern matching, Methods Enzymol. 183: 403-427, Eddy, S.R. (1996): Hidden markov models. Curr. Opin. Strct. Biol. 6: 361-365, Eddy, S.R. (1998): Profile hidden markov models. Bioinformatics. 14: 755-763, Gribskov, M. McLachlan, A.D. und Eisenberg D. (1987): Profile analysis: Detection of distantly related proteins. Proc. Natl. Acad. Sci. USA. 84: 4355-5358, Heinikoff, S. (1996): Scores for sequence searches and alignment, Curr; Opin. Strct. Biol. 6: 353-360, Krogh, A., Brown, M., Mian, I.S., Sjolander, K. und Haussler, D. (1994): Hidden markov models in computational biology: Applications to protein modelling. J. Mol. Biol. 235: 1501-1531, Taylor, W.R. (1986): Identification of protein sequence homology by consensus template alignment. J. Mol. Biol. 188: 233-258.

[0176] In general the sequence are selected such that a query using a search sequence returns a result consisting of sequences which are at least to a certain degree similar to the query sequence.

[0177] In one embodiment of the invention amino acid sequences of the present invention are used to model the three-dimensional structure of the protein. Initially, this involves the comparison of the protein sequence with the sequence of related proteins where the structure is known, such as the human PPAR γ ligand-binding domain (Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, Rosenfeld MG, Willson TM, Glass CK, Milburn MV, Nature 1998 Sep 10;395(6698):137-43). The three-dimensionale structure can then be modelled using computer programs. From the three-dimensional structure, binding sites of potential inhibitors or activators can be predicted. It can further be predicted which kinds of molecule might bind there. The predicted substances can then be screened to test their effect on nuclear receptor activity.

[0178] The present invention shall now be further described in the following example with respect to the accompanying sequence protocol. In the sequence protocol,

SEQ ID NO. 1 depicts the nucleotide sequence encoding for a polypeptide according to the present invention, and SEQ ID NO.2 depicts the amino acid sequence of the polypeptide according to the present invention.

EXAMPLES

EXAMPLE 1: CLONING AND EXPRESSION OF THE GENE ACCORDING TO THE INVENTION

[0179] Construction of suitable vectors containing the desired coding and control sequences employs standard ligation and restriction techniques that are well understood in the art. Isolated plasmids, DNA sequences, were synthesised oligonucleotides were cleaved, tailored, and religated in the form desired.

[0180] Site-specific DNA cleavage was performed by treatment with the suitable restriction enzyme (or enzymes)

under conditions that are generally understood in the art, and the particulars of which are specified by the manufacturer of these commercially available restriction enzymes.

[0181] See, e.g., New England Biolabs, Product Catalog. In general, about 1 µg of plasmid and/or DNA sequence was cleaved by one unit of enzyme in about 20 µl of buffer solution. Often excess of restriction enzyme was used to ensure complete digestion of the DNA substrate. Incubation times of about one hour to two hours at about 37°C are workable, although variations are tolerable.

[0182] After each incubation, protein was removed by extraction with phenol/chloroform, and may be followed by ether extraction. The nucleic acid was recovered from aqueous fractions by precipitation with ethanol. If desired, size separation of the cleaved fragments was performed by polyacrylamide gel or agarose gel electrophoresis using standard techniques. A general description of size separations is found in *Methods in Enzymology* 65, 499-560 (1980).

[0183] Transformed host cells are cells which have been transformed or transfected with recombinant expression constructs made using recombinant DNA techniques and comprising mammalian nuclear receptor LXR-α3 encoding sequences. Preferred host cells for transient transfection are COS-7 cells. Transformed host cells may ordinarily express nuclear receptor LXR-α3, but host cells transformed for purposes of cloning or amplifying nucleic acid hybridisation probe DNA need not express the nuclear LXR-α3 receptor. When expressed, the mammalian nuclear LXR-α3 receptor protein was typically located in the host cell membrane.

[0184] Cultures of cells derived from multicellular organisms are desirable hosts for recombinant nuclear receptor LXR-α3 protein synthesis. In principal, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. However, mammalian cells are preferred. Propagation of such cells in cell culture has become a routine procedure. See *Tissue Culture* (Academic Press, Kruse & Patterson, Eds., 1973). Examples of useful host cell lines are bacteria cells, insect cells, yeast cells, human 293 cells, VERO and HeLa cells, LMTK-cells, and WI138, BHK, COS-7, CV, and MDCK cell lines. Human 293 cells are preferred.

EXAMPLE 2: NUCLEAR RECEPTOR LXR-α3 cDNA ISOLATION FROM HUMAN AND OTHER ORGANISMS:

[0185] A cloning strategy was used to clone the LXR-α3 receptor cDNA from specific cDNA libraries (Clontech, Palo Alto) or alternatively, RNA was obtained from various tissues and used to prepare cDNA expression libraries by using for example an Invitrogen kit. (Invitrogen Corporation, San Diego). For the isolation of the LXR-α3 cDNA clone the chosen library was screened under stringent condition (see definitions above) by using an LXR specific probe. The cDNA insert of positive clones was subsequently sequenced and cloned in a suitable expression vector.

[0186] Additionally, a full length receptor LXR-α3 clone from human was obtained by using RACE PCR technology. In brief, suitable cDNA libraries were constructed or purchased. Following reverse transcription, the first strand cDNA was used directly in RACE PCR reactions using a RACE cDNA amplification kit according to the manufactures protocol (Clontech, Palo Alto). Amplified fragments were purified, cloned and subsequently used for sequence analysis.

[0187] To obtain information about the genomic organisation of the receptor gene, genomic libraries were screened (commercially available clone libraries were used) with a receptor specific probe under stringent conditions. Positive clones were isolated and the complete DNA sequence of the putative receptor region was determined by sequence analysis (Maniatis et al., *Molecular Cloning: A laboratory Manual*, Cold Spring Harbor Laboratory Press, N.Y.(1989)) (human genomic clone deposited at "Deutsche sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, International Depository Authority under the Budapest Treaty in Germany) under number DSM 14483).

EXAMPLE 3: NUCLEAR RECEPTOR LXR-α3 LIGAND BINDING ASSAY:

[0188] Saturation ligand binding analysis and ligand competition studies are carried out by using an over expressed LXR-α3 receptor-protein which is incubated with a labelled potential ligand at different concentrations. The LXR-α3 receptor may be immobilised on suitable surfaces. Various competitors are added in the presence of a labelled ligand. Bound and unbound ligand is separated by using e.g. gel filtration or charcoal based methods or simply removed by a washing step if the receptor protein LXR-α3 is immobilised. Receptor-bound ligand is detected by scintillation counting.

EXAMPLE 4: FORMATION OF LXR-α3-RXR OR OTHER PROTEIN COMPLEXES

[0189] In order to explore the functional properties of LXR-α3, the DNA binding properties of LXR-α3 are analyzed. It has previously been shown that RXR is a common heterodimeric partner of various members of the nuclear receptor superfamily required for high affinity DNA binding (Hallenbeck et al., *PNAS* 89: 5572-5576 (1989)), Kliever et al., *EMBO J.* 11: 1419-1435 (1992)). It has also been shown that DNA and ligand binding activities of the *Dros. melanogaster* ecdyson receptor (EcR) require heterodimer formation with RXR or USP (the homologue of RXR) (O'Malley in *Endocrinology* 125: 1119-1120 (1989)).

[0190] Consequently, it is of interest whether LXR- α 3 can interact with RXR, or with other members of the family.

[0191] A two hybrid system is used. CV-1 cells are transiently transfected with cytomegalovirus promoter driven expression vectors containing the yeast GAL4 DNA binding domain (DBD) alone, GAL4 linked to LXR- α 3 LBD (LBD; i.e. GAL4-LXR- α 3) and the 78 amino acid Herpes virus VP16 transactivation domain (VP) linked to the amino terminal end of the LBDs for human (or mouse) RXR α (VP-RXR), mouse PPAR γ (VP-PPAR) VDR (VP-VDR) and others.

[0192] All cells are cotransfected with a luciferase reporter construct containing about 4 copies of the yeast GAL4 upstream activating sequence and a β -galactosidase expression vector as internal control.

[0193] CV-1 cells are grown in DMEM supplemented with 10% AG1-X8 resin-charcoal stripped calf bovine serum, 50 U/ml penicillin G and 50 μ g/ml streptomycin sulfate (DMEM-CBS) at 37°C in 5% CO₂. One day prior to transfection, cells are plated to 50-80% confluence using a phenol-red free DMEM with 10% resin charcoal stripped fetal bovine serum (DMEM-FBS). Cells are transfected (with reporter construct (300 ng/10⁵ cells), cytomegalovirus driven receptor (100 ng/10⁵ cells) and β -galactosidase expression vectors (500 ng/10⁵ cells) by lipofection using N- {2-(2,3)-dioleoyloxy}propyl-N,N,N-trimethyl ammonium methyl sulfate} according to the manufacturer's instructions (DOTAP, Boehringer Mannheim).

[0194] After 2 hours the liposomes are removed and cells treated for 40 hours with phenol-red free DMEM-FBS containing farnesol as the ligand. Cells are harvested and assayed for luciferase and β -galactosidase activity.

[0195] All points are preferentially performed in triplicate and should ideally vary less than 10%. Experiments are repeated a 2-4 times. Data points are normalized for differences in transfection efficiency using β -galactosidase, and plotted as relative activity where the untreated reporter is defined to have an activity of 1 unit.

[0196] Neither the GAL4 DBD, nor the GAL4-LXR- α 3 chimera should be capable of stimulating transcription from a reporter construct containing the GAL4 upstream activating sequence. Similarly, a fusion protein containing the Herpes virus VP16 transactivation domain linked to the RXR α -LBD (VP-RXR) should be inactive when expressed alone or with the GAL4 DBD. However, when GAL4-LXR- α 3 and VP-RXR are coexpressed, the reporter could be activated, indicating that LXR- α 3 and RXR α interact efficiently in cells. Using similar VP16-LBD fusion proteins, interaction can be tested between LXR- α 3 and receptors for peroxisome proliferators/fatty acids (PPAR), vitamin D₃ (VDR), thyroid hormone, (T₃R), retinoic acid (RAR), or other members of the nuclear receptor superfamily (see also US Pat. 6.005.086).

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 <213> homo sapiens

<400> 2

EP 1 371 662 A1

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5 Ala Val Glu Leu Trp Lys Pro Gly Ala Gln Asp Ala Ser Ser Gln Ala
20 25 30

10 Gln Gly Gly Ser Ser Cys Ile Leu Arg Glu Glu Ala Arg Met Pro His
35 40 45

Ser Ala Gly Gly Thr Ala Gly Val Gly Leu Glu Ala Ala Glu Pro Thr
50 55 60

15 Ala Leu Leu Thr Arg Ala Glu Pro Pro Ser Glu Pro Thr Gly Val Leu
65 70 75 80

20 Ser Glu Glu Gln Ile Arg Leu Lys Lys Leu Lys Arg Gln Glu Glu Glu
85 90 95

Gln Ala His Ala Thr Ser Leu Pro Pro Arg Ala Ser Ser Pro Pro Gln
100 105 110

25 Ile Leu Pro Gln Leu Ser Pro Glu Gln Leu Gly Met Ile Glu Lys Leu
115 120 125

30 Val Ala Ala Gln Gln Gln Cys Asn Arg Arg Ser Phe Ser Asp Arg Leu
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Arg Val Thr Pro Trp Pro Met Ala Pro Asp Pro His Ser Arg Glu Ala
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35 Arg Gln Gln Arg Phe Ala His Phe Thr Glu Leu Ala Ile Val Ser Val
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50 Thr Phe Leu Lys Asp Phe Ser Tyr Asn Arg Glu Asp Phe Ala Lys Ala
225 230 235 240

Gly Leu Gln Val Glu Phe Ile Asn Pro Ile Phe Glu Phe Ser Arg Ala
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EP 1 371 662 A1

Met Asn Glu Leu Gln Leu Asn Asp Ala Glu Phe Ala Leu Leu Ile Ala
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Ile Ser Ile Phe Ser Ala Asp Arg Pro Asn Val Gln Asp Gln Leu Gln
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Val Glu Arg Leu Gln His Thr Tyr Val Glu Ala Leu His Ala Tyr Val
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Ser Ile His His Pro His Asp Arg Leu Met Phe Pro Arg Met Leu Met
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Lys Leu Val Ser Leu Arg Thr Leu Ser Ser Val His Ser Glu Gln Val
325 330 335

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Phe Ala Leu Arg Leu Gln Asp Lys Lys Leu Pro Pro Leu Leu Ser Glu
340 345 350

Ile Trp Asp Val His Glu
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Claims

1. A polypeptide of LXR alpha receptor, comprising the amino acid sequence according to SEQ ID No. 2, or having an amino acid sequence substantially the same or similar as SEQ ID No. 2.
2. A fusion protein comprising a polypeptide according to claim 1.
3. A polypeptide according to claim 1 or 2, wherein the polypeptide is a synthetically produced polypeptide.
4. A nucleic acid encoding for a polypeptide according to any of claims 1 to 3, wherein said nucleic acid is genomic DNA, cDNA, immature mRNA or mature mRNA, in particular the nucleic acid sequence according to SEQ ID No. 1, optionally comprising a label.
5. A nucleic acid according to claim 4, wherein the sequence of the nucleic acid comprises at least one intron and/or a polyA-sequence.
6. A nucleic acid according to claim 4 or 5 in form of its complementary antisense-sequence.
7. A nucleic acid according to any of claims 4 to 6, wherein the nucleic acid is a synthetically produced nucleic acid.
8. A vector, in particular in form of a plasmid, shuttle vector, phagemid, cosmid, expression vector and/or vector for gene therapy, comprising a nucleic acid according to any of claims 4 to 7.
9. A host cell, comprising a vector according to claim 8.
10. A host cell according to claim 8 or 9, wherein the host cell expresses a polypeptide according to any of claims 1 to 3.
11. A method for the production of an antibody, in particular a monoclonal or polyclonal antibody, directed against a polypeptide according to any of claims 1 to 3, wherein an anti-body producing organism is immunised with a polypeptide according to any of claims 1 to 3.
12. An antibody produced according to claim 11, **characterised in that** the antibody is directed against a polypeptide according to any of claims 1 to 3.
13. A method for screening for agents which are capable of inhibiting the cellular function of the LXR alpha receptor, comprising the steps of:
 - contacting one or more candidate agents with a polypeptide according to any of claims 1 to 3,
 - removing unbound agent(s), and
 - detecting, whether the agent(s) interact with the polypeptide of the nuclear receptor.
14. A method for inhibiting the cellular function of the LXR alpha receptor, comprising the steps of:
 - contacting a cell with a binding agent of a polypeptide according to any of claims 1 to 3, whereby the cellular function of LXR alpha receptor is inhibited.
15. A method according to claim 14, **characterised in that** the binding agent is selected from the group comprising an antibody, RNA, an antisense oligonucleotide, and a ribozyme.
16. A method according to claim 14, **characterised in that** the cell is in a body.



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Application Number

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

EP 02 01 3109

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			C07K C12N A61K A01K
INCOMPLETE SEARCH The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims. Claims searched completely : Claims searched incompletely : Claims not searched : Reason for the limitation of the search: Although claims 14-16 can be considered as a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.			
Place of search		Date of completion of the search	Examiner
MUNICH		30 August 2002	Tudor, M
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

EPO FORM 1503 03.92 (P04-007)



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